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Isolation and Long-Term Culture of Rat, Rabbit, and Human Nasal Turbinate Epithelial Cells

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16. ABSTRACT

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JOURNAL ARTICLE

ISOLATION AND LONG-TERM CULTURE OF RAT, RABBIT, AND HUMAN NASAL TURBINATE EPITHELIAL CELLS

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SUMMARY

Nasal turbinate epithelial cells were isolated from rats, rabbits, and humans using either a surgical or an in situ enzyme incubation technique. The culture conditions that permit optimal cell attachment and selective growth of the nasal epithelial cells were determined. These conditions will permit the long-term culture of these cells where typically 20 to 30 population doublings were observed. Differences between rat and human nasal epithelial cells were seen in substrate requirements, colony-forming efficiency, and response to fetal bovine serum and bovine serum albumin. These methodology and results will permit mechanistic studies of normal and abnormal cellular function and comparative response studies between nasal epithelial cells from rats and humans.

Key words: nasal epithelium; human epithelial cell culture; rat epithelial cell culture; rabbit epithelial cell culture; respiratory tract cell culture; cell isolation procedures.

INTRODUCTION

The nasal cavity is a target site for the deposition and cytotoxic effects of many environmental pollutants. Animal studies have shown that inhalation of certain toxic substances such as formaldehyde or acrolein can cause acute and chronic pathologic disorders of the nasal cavity (1,5-7,11-13). To quantitate and study in detail the cytotoxic and preneoplastic changes that occur in the lining of the upper respiratory tract, cell culture methodology needed to be developed.

Although recent studies have utilized short-term (<36 h) cultures of rat nasal turbinate explants or cells (2-4,8) to study metabolism and DNA damage from nasal toxicants, much longer culture survival times are necessary to study the more chronic effects of nasal toxicants.

Wu et al. (16) have described a method for isolating and culturing human nasal turbinate epithelial (NTE) cells. In the paper presented here, two methods are described for the isolation of NTE cells from rat and rabbit and an explant method for the isolation of human NTE cells. Also described is the development of the long-term NTE culture methodology necessary for future studies of chronic cytotoxicity and carcinogenesis.

MATERIALS AND METHODS

Rat and rabbit surgical isolation of cells. Two methods were developed to collect epithelial cells lining the nasal cavity of 8 to 12-wk-old rats (male F-344, Charles River, Wilmington, MA) and 4 to 8-wk-old rabbits (male, New Zealand White, Charles River). The first was a surgical technique (*see* Fig. 1) in which the lateral, maxillary, and ethmoid turbinates were isolated. The rats were killed by CO_2 asphixyation, and the rabbits were killed by an overdose of Nembutal (Abbott Laboratories, North Chicago, IL) injected into the ear vein. The surgical procedure for both species included removing the skin from the head and using bone cutters or surgical scissors to make a lateral incision from the nares to the orbital sinuses. The frontal bone was then removed by cutting along the lateral fissure. The turbinates from each region were teased off the supporting bone using forceps and scissors and collected into separate tubes, each containing Joklik's modified minimum essential medium (MEM), (GIBCO, Grand Island, NY), and 100 μg/ml gentamicin (U.S. Biochemical Corp., Cleveland, OH). This rinse media was removed and an enzyme mixture, consisting of a protease, collagenase, and hyaluronidase, was added to dissociate the cells from the tissue. These enzymes were obtained from Sigma Chemical Co., St. Louis, MO. The enzyme mixture, prepared before surgery, was kept on ice while going into solution, then warmed to room temperature for incubation. The tissue was incubated with the enzyme mixture at room temperature for 30 min, then gently vortexed to dislodge the cells from supporting stroma. The dissociated cells and media were transferred to a new tube on ice, and fetal bovine serum (FBS) (Sterile Systems, Inc., Logan, UT) was added to the cells to a final concentration of 10%. This incubation procedure was repeated with each group of tissue for another 30 min to maximize the cell yield.

Cells from the two incubations were pooled and pelleted by centrifugation (1000 rpm/10 min), then resuspended in "attachment" medium: Ham's F12

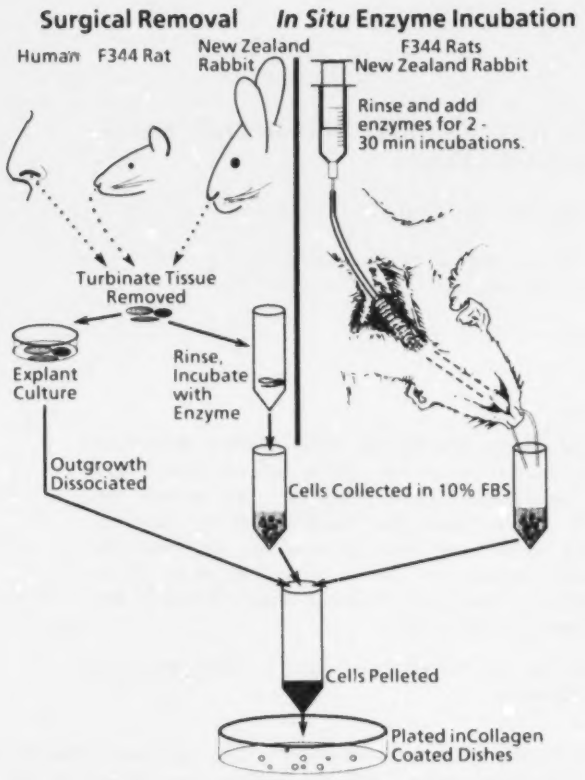


FIG. 1. Diagrammatic representation of nasal turbinate epithelial cell isolation procedures: surgical and in situ incubation. The details are described in Materials and Methods.

(GIBCO) with six growth factors (6GF), which are described in Table 1, plus 10% FBS. The F12 + 6GF medium was originally described by Wu et al. (16) for the growth of human NTE cells. Cell numbers were determined by hemacytometer counting. Trypan blue dye was then added to determine viability. Cells were seeded onto collagen-coated, plastic tissue culture dishes (10). Cells were allowed to attach for 24 h, and the attachment medium was replaced with culture medium. For the rat cells, the culture medium included Ham's F12, 6GF, and 300 μg/ml bovine serum albumin (BSA) (Sigma Chemical Co.). The culture medium for the rabbit cells was Ham's F12:3T3-conditioned Dulbecco's modified Eagle's medium (DMEM) (4:1) plus 6GF. The 3T3-conditioned DMEM was prepared by growing 3T3 fibroblasts to 80% confluence then conditioning DMEM + 2% FBS at 20 ml/75-cm² flask for 3 d. This medium was removed and filter sterilized before use. Cultures were refluided twice a week and maintained in a 5% CO_2:95% air incubator.

Rat and rabbit in situ incubation procedure. The second cell collection procedure involved an in situ incubation of the enzyme mixture (*see* Fig. 1). The animals were killed as described earlier, and the tracheas were surgically exposed and separated from the esophagus. A small incision was made between the tracheal rings near the larnyx. Intramedic polyethylene tubing (Clay Adams) was used to cannulate the trachea and nares for instilling and removing the medium or enzyme mixture into and from the nasal cavity (Fig. 2). A suture was tied tightly around the trachea to secure the tube. The esophagus was sealed with another suture, and the pharyngeal cavity was sealed with a polyether impression material (Impregum, ESPE, West Germany). The two latter procedures were to ensure that the enzyme mix only entered the nasal cavity.

The nasal cavity was rinsed with Joklik's modified MEM with 100 μg/ml gentamicin, using a needle and syringe inserted into the tracheal tubing. The nasal cavity was then filled with the same enzyme mixture as that used in the above surgical procedure (approximately 1 ml/rat and 5 ml/rabbit), and allowed to incubate for 0.5 h at room temperature.

At the end of the incubation, 20 to 50 ml of warm (37° C) Joklik's MEM and 100 μg/ml gentamicin were forcibly flushed through the tracheal tube and out the nostrils. The cells and medium were collected in a sterile centrifuge tube and placed on ice. FBS was immediately added to a final concentration of 10% to inhibit further enzyme activity.

The incubation procedure was repeated by adding 37° C enzyme mixture to the nasal cavity for another 30 min. Cells from both rinses were pooled and pelleted by centrifugation at 1500 rpm for 15 min. Cells were then counted and plated as previously described.

Explant culture. The human nasal turbinate epithelial cell cultures were prepared from surgical specimens (lateral turbinates from nasal reconstructions) using an explant outgrowth cell culture system. The tissue was cut in a sterile hood into 0.5-cm² pieces and placed in plastic tissue culture dishes containing 2 to 3 ml of culture medium—Ham's F12 and 6GF. When the explant outgrowths were 1 to 2 cm² in diameter, the explants were transplanted onto new dishes. The remaining outgrowth of cells was rinsed with calcium and magnesium-free (CMF) Hanks' balanced salt solution, and dissociated with 0.05% trypsin-0.02% EDTA (GIBCO) in CMF Hanks' for 5 min. The dissociated cells were then pooled, pelleted by

TABLE 1

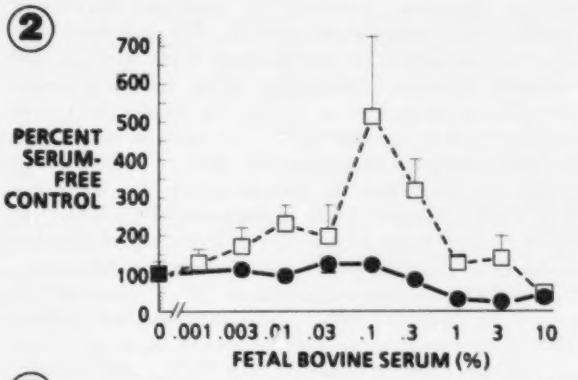
GROWTH FACTORS FOR RAT, RABBIT, AND HUMAN NASAL TURBINATE CELL CULTURE

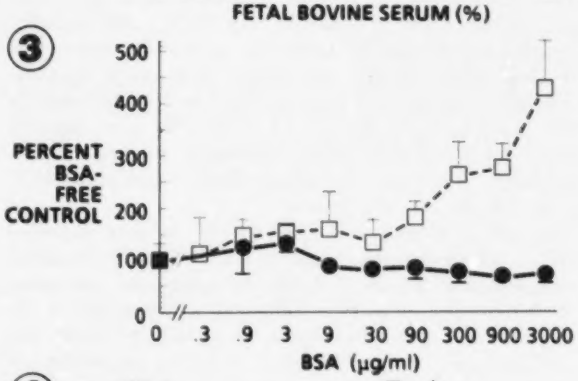
Growth Factor[a]	Concentration
Insulin, from bovine pancreas	2.5 μg/ml
Transferrin, human, 98%	2.5 μg/ml
Hydrocortisone, crystalline	1.0 μ*M*
Cholera Toxin, from *Vibrio cholerae*	20.0 ng/ml
Epidermal growth factor, from mouse submaxillary glands	12.5 ng/ml
Bovine hypothalamus extract[b]	0.4%

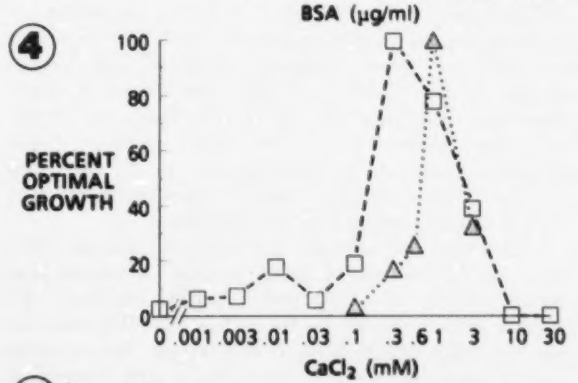
[a]The first five growth factors were obtained from Sigma Chemical Co.

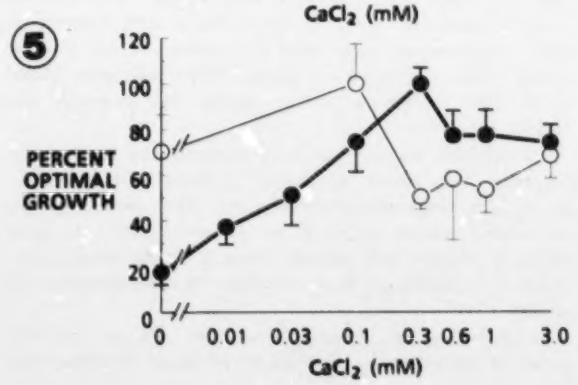
[b]The bovine hypothalamus extract was prepared (9) from tissue obtained from Rockland, Inc., Philadelphia, PA.

centrifugation, resuspended, counted, and plated for the various growth assays. Cultures were routinely refluided twice a week.









Optimizing culture conditions. Cells from each species were collected as described earlier and seeded at 2 to 5 × 10^4/60-mm dish for optimizing culture conditions.

The growth substrates tested were tissue culture plastic, glass, collagen film (600 μg/dish), collagen (15 μg/dish) + fibronectin (10 μg/dish) + BSA (10 μg/dish) film, and Primeria dishes (Falcon, Oxnard, CA).

Optimum levels of calcium, FBS, and BSA were determined by testing 10 half-log doses. For the calcium studies, calcium-deficient Ham's F12 medium was used (GIBCO). Conditioned medium (for rabbit cultures) was treated with chelating resin (Sigma) to remove calcium. Calcium was then added back to cultures in the form of calcium chloride.

Attachment media were compared by seeding primary cells into normal culture medium plus 0 to 10% FBS for 24 h and then changing the media to normal culture media alone.

The experiments were terminated after 7 to 14 d by dissociating the cells with trypsin (0.05%)-EDTA (0.02%), 2 ml/dish for 5 to 10 min. Total cell numbers were determined from hemacytometer counts, and viability was measured by trypan blue exclusion.

FIG. 2. Growth of rat (□) and human (●) NTE cells in fetal bovine serum. Cells were isolated using the in situ procedure and plated at 2 × 10^4 into 60-mm collagen-coated dishes. Rat cells were plated in culture media containing 10% FBS for cell attachment for 24 h, then media was changed to Ham's F12 + 6F + FBS. Human cells were plated directly into Ham's F12 + 6F + FBS. Total cell numbers were determined after 7 d in culture and compared here to serum-free controls. The mean of the serum-free control cell numbers for human NTE cells was 2.6 × 10^5 and for rat NTE cells was 0.6 × 10^5 cells/dish.

FIG. 3. Growth of rat (□) and human (●) NTE cells in bovine serum albumin. Cells were isolated using the in situ procedure and plated at 2 × 10^4 into 60-mm collagen-coated dishes. Rat cells were plated into culture media containing 10% FBS for cell attachment for 24 h. The media was then changed to Ham's F12 + 6F + BSA. Human cells were plated directly into Ham's F12 + 6F + BSA. Total cell numbers were determined after 7 d in culture and compared to BSA-free controls. The mean of the BSA-free control cell number for human NTE cells was 2.6 × 10^5 and for rat NTE cells was 0.6 × 10^5 cells/dish.

FIG. 4. Effects of calcium concentration on the growth of rat (□) and rabbit (▲) NTE cells. Cells were isolated using the in situ procedure and plated at 2 × 10^4/60-mm collagen-coated dishes in Ham's F12 + 6F + 10% FBS for cell attachment. After 24 h dishes were rinsed and refluided with either calcium-free Ham's F12 + 6F + 300 μg/ml BSA (for rat cells) or conditioned media (for rabbit cells). The conditioned media used in the rabbit culture media was treated with chelating resin to remove calcium ions. Calcium was added to media at half-log concentrations from 0.001 to 30 m*M*. Total cell numbers were determined on Day 7 and the data standardized as percent of optimal growth. Optimal growth for rat NTE cells was 2.2 × 10^5/60-mm dish in this experiment and for rabbit cells was 8 × 10^4 cells/60-mm dish.

FIG. 5. Effects of calcium concentration on the growth of human cells. Cells were collected from two samples using the explant culture procedure and plated at 2 × 10^4 cells/60-mm dish. After 24 h in Ham's F12 + 6F, the media was changed to calcium-free Ham's F12 + 6F and $CaCl_2$ was added to the media at half-log concentrations from 0.001 to 30 m*M*. Total cell numbers were determined on Day 7 and the data were standardized as percent of optimal growth. Sample 1 (○); sample 2 (●). Optimal growth for sample 1 was 1.75 × 10^5 cells/60-mm dish and for sample 2 was 6.4 × 10^4 cells/60-mm dish.

TABLE 2

COMPARATIVE GROWTH OF RABBIT NASAL EPITHELIAL CELLS IN VARIOUS MEDIA

Culture Media	Percent FBS in Attachment Media[a]	No. Colonies/ 10^5 cells
1. F12:DMEM(1:1) + 6F[b] + 0.01% FBS	10	42
2. F12:DMEM(1:1) + 6F + 0.01% FBS	5	26
3. F12:DMEM(1:1) + 6F	10	54
4. F12:CM[c](4:1) + 6F	10	TNTC[d]
5. F12:DMEM(4:1) + 6F + 0.2% FBS	10	TNTC
6. F12:DMEM(1:1) + 6F	5	36

[a] Present during the first 24 h after plating.
[b] See Table 1.
[c] CM is 3T3-conditioned DMEM + 2% FBS media.
[d] TNTC = too nunerous to count.

Determination of population doubling time. Population doubling times were determined by seeding 2×10^4 viable cells per dish and counting total cells at 2 to 3-d intervals for 14 d. Population doubling times were calculated from the slope of the exponential portion of growth curves.

Colony-forming efficiencies (CFE) were determined by plating 10^4 to 5×10^5 cells/dish, and after 7 d fixing and staining the colonies with methylene blue (Fisher Scientific, Pittsburgh, PA). Colonies of eight or more cells were enumerated using a dissecting scope or an automatic colony counter (Artek Systems Corp., Farmingdale, NY).

The epithelial origin of the cells was verified by the use of a peroxidase-antiperoxidase (PAP) staining technique (DAKO PAP Kit, DAKO Corporation, Santa Barbara, CA) which identifies cellular keratin proteins using soluble PAP-immune complexes.

Results

Cell isolation procedures. Two techniques were developed to isolate NTE cells from the rat and rabbit, a surgical procedure and an in situ incubation technique.

TABLE 3

COMPARATIVE GROWTH OF RAT AND HUMAN CELLS ON DIFFERENT SUBSTRATA

	Percent Relative Growth[a]			
NTE Cells	TC Plastic	Collagen Film[b]	Glass	Primeria[c]
Rat	100	287	70	75
Human	100	445	86	ND[d]

[a] Relative to uncoated tissue culture plastic dishes.
[b] Tissue culture dishes with a collagen film.
[c] Primeria dishes are a trademark of Falcon Plastics, Inc.
[d] ND = not determined.

To isolate cells from specific regions of the nasal cavity, such as the lateral or maxillary turbinates, a surgical procedure was used. Types VI and XIV proteases from Sigma Chemical Company, collagenase, and hyaluronidase, and mixtures of these were tested in various digestion protocols to optimize cell yield, viability, and reproductive survival. The proteases were tested at concentrations ranging from 0.1% to 0.5% and compared to mixtures containing either 0.1% collagenase or 0.1% hyaluronidase or both. The tissues were then incubated either overnight at 4° C or at room temperature for two consecutive 30-min periods. From a series of these studies, we found that the optimal enzyme mix was 0.5% Type XIV protease, 0.1% collagenase (Type IV), and 0.1% hyaluronidase (Type IV-S). Digestion of rat and rabbit nasal turbinate tissue with this enzyme mixture for two half-hour incubation periods at 22° C produced the most reproductively viable epithelial cells for culture. This methodology was tested numerous times using rats and rabbits. The number of rat NTE cells obtained usually ranged from 10^5 to 10^6 for each of the three nasal regions. The number of rabbit NTE cells typically isolated by this method was between 1 to 5×10^6 cells. In five experiments, 1 to 5×10^5 NTE cells were obtained from each human nasal turbinate specimen when incubated with Type VI protease (Sigma) overnight at 4° C as described by Wu et al. (15).

A second method was developed to increase epithelial cell yield and reduce red blood cell and fibroblast contamination. Figure 1 shows the operational procedure of the in situ incubation technique as described in the methods and materials section. Various incubation and washing procedures were tested including prerinsing; 30 to 60-min enzyme incubation periods at either 4° or 37° C; one, two, or three incubation periods; and various amounts of pronase (0.1 to 0.5%). The optimal collection procedure was found to be two consecutive 30-min incubations with 0.5% Type XIV protease, 0.1% collagenase, and 0.1% hyaluronidase at 37° C. This procedure in over 10 assays yielded approximately 0.5 to 1 $\times 10^6$ cells/rat and 0.5 to 1×10^7 cells/rabbit.

Establishment of primary cell cultures. Human NTE cells can be dissociated from surgical specimens and seeded directly into complete growth medium (16). Optimal cell attachment for rat and rabbit NTE cells was observed when the cells were first plated into complete medium plus FBS for 2 d. Typically, a 20% increase in CFE was observed when cells were plated in 5% FBS as compared to a serum-free control. When cells were plated in a 10% serum-containing media the increase was 10-fold.

To establish multiple primary cultures and expand cell numbers from small specimens of human nasal tissue, an explant outgrowth procedure was also used. Explants have been serially replated up to seven times to yield multiple primary cell cultures from a single tissue piece. Further replanting has resulted in diminished cell growth.

Optimization of growth conditions. In an iterative series of experiments, five kinds of basic medium were

TABLE 4

COLONY FORMING EFFICIENCIES OF RAT, RABBIT, AND HUMAN NTE CELLS AT VARIOUS SEEDING DENSITIES

Seeding Density[a]	Colony Forming Efficiency (%)[b]		
	Rat	Rabbit	Human[c]
1×10^3	ND[d]	ND	0.00
5×10^3	ND	ND	1.22
1×10^4	0.94	ND	1.02
2×10^4	1.11	ND	TNTC[e]
5×10^4	0.81	0.11	TNTC
1×10^5	0.41	0.05	TNTC
2×10^5	0.21	0.02	TNTC
5×10^5	ND	0.01	ND

[a] Number of viable cells seeded per 60-mm dish.
[b] Number of colonies/seeding density after 10 d.
[c] Human cells were grown in 1 m*M* calcium to induce colony formation.
[d] ND = not determined.
[e] TNTC = too numerous to count.

tested: Ham's F12, DMEM, 3T3-conditoned DMEM, Medium 199, and Waymouth's 752/1. These were tested with different combinations of the growth factors listed in Table 1, and with 0.01 to 3% FBS. In some cases, increased FBS (5 or 10%) was added during the first 24 h of culture to increase cell attachment. A typical experiment is shown in Table 2. For rat and human NTE cells, a serum-free Ham's F12 plus insulin, epidermal growth factor, bovine hypothalamus extract, cholera toxin, hydrocortisone, and transferrin provided optimal growth. This media mixture was initially developed by Dr. Reen Wu and coworkers (16) for human nasal turbinate epithelial cells. The optimal complete medium for rabbit NTE cells was a 4:1 mixture of Ham's F12 and 3T3 conditioned DMEM/2% FBS plus the six growth factors previously listed. The final FBS concentration was 0.4% in this medium.

Previous studies with primary tracheal epithelial cell cultures from various species showed that cells from different species required different substrata for optimal growth (14). In a series of comparative experiments, uncoated tissue culture dishes, collagen-coated dishes, and glass dishes were examined for their ability to provide optimal attachment and growth for rat and human NTE cells. Rat and human NTE cells will attach and grow to uncoated tissue culture dishes and glass dishes, but a three- to fivefold enhancement was seen when these cells were plated into a dish coated with a collagen film (Table 3). Rabbit NTE cells seemed to grow equally well on uncoated or collagen-coated dishes, and to a lesser extent on glass (unpublished observations).

To maximize plating efficiency for the three NTE cell types, the CFE was determined at various seeding densities. As seen in Table 4, plating densities in the range of 1 to 5×10^4 cells/60-mm dish provided high plating efficiencies. Rat or human NTE cells usually had a higher CFE compared to rabbit.

Nasal turbinate epithelial cells from rats and humans were next plated in media containing various amounts of FBS. Figure 2 shows a comparison of rat and human cell growth as a function of serum concentration in the media. Rat NTE cell growth was stimulated fivefold at 0.1% FBS, whereas human NTE cells were only stimulated 1.25-fold at the same concentration. At higher FBS concentrations the growth of human NTE cells was severely inhibited.

Rat and human cells in medium containing different concentrations of BSA also grew at different rates (Fig. 3). The growth of rat NTE cells increased as the amount of BSA present in the media increased. A fourfold increase in cell number was observed at a BSA concentration of 3 mg/ml compared to BSA-free media. At the higher concentrations of BSA, fibroblast growth was minimal compared to high FBS conditions. In contrast to rat NTE cultures, 3 mg/ml BSA stimulated human NTE cell growth by only 1.3-fold compared to BSA-free media, then the grown decreased steadily at higher concentrations to below control levels. The following experiments were performed to optimize the calcium concentration for NTE cell culture. These experiments also displayed a species-dependent response (Fig. 4). Rat NTE cells grew optimally at 0.3 m*M* calcium whereas rabbit cells grew optimally at a concentration of 1.0 m*M*. Optimal human NTE cell growth varied between 0.1 and 1.0 m*M* calcium depending on individual tissue sample (Fig. 5).

For cells grown in optimized media the growth rates were quantitated in terms of population doubling times. Table 5 shows the mean population doubling times of rat, rabbit, and human NTE cells. Rat and human NTE cells grew at similar rates with the cell number doubling approximately every 60 to 70 h. Rabbit NTE cell cultures grew at a much faster rate, doubling in number approximately every 24 h. In several cases, rat, rabbit, and human primary cell cultures were passaged at confluence successfully four to five times. From the growth curves it was estimated that five to six population doublings occurred per passage. Therefore the apparent life span of these cultures is estimated to be 20 to 30 population doublings.

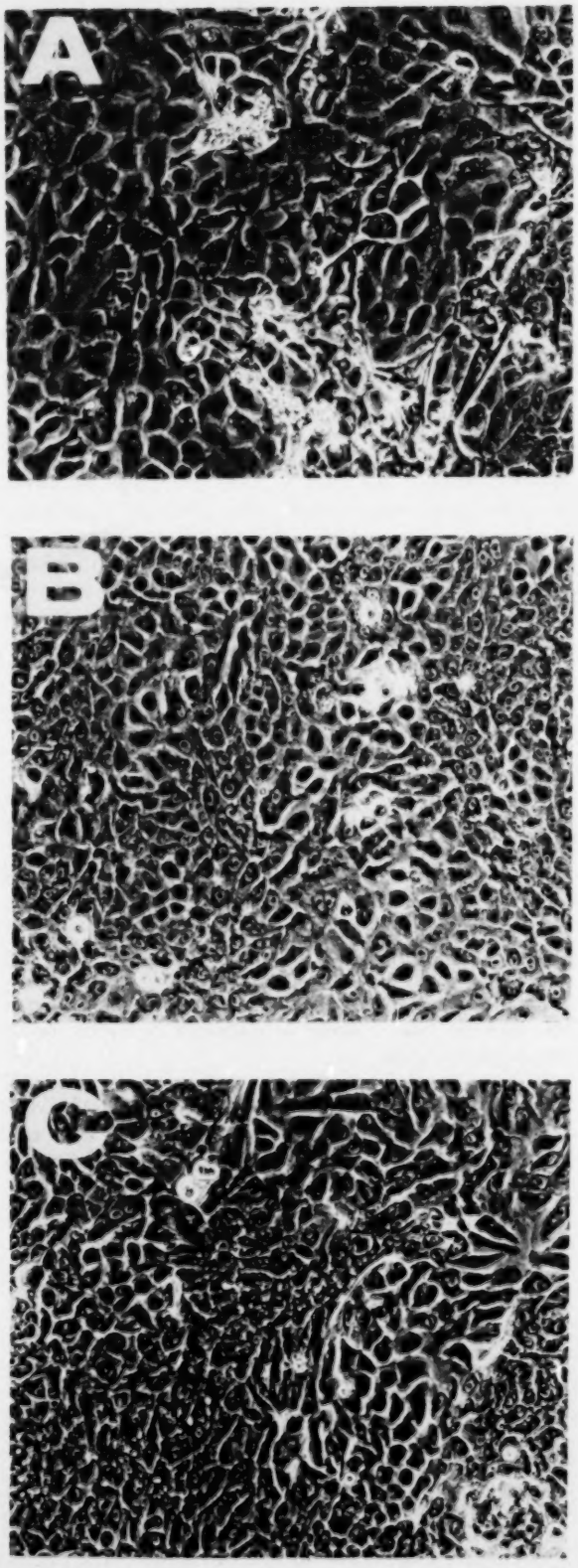
Cellular morphology. The morphology of the rat NTE cells in culture was typically epithelial in nature. The cells were polygonal in shape, with some areas that were multilayered and contained keratinizing squamous epi-

TABLE 5

COMPARATIVE GROWTH RATES OF RAT, RABBIT, AND HUMAN NASAL TURBINATE EPITHELIAL CELLS IN OPTIMIZED CULTURE CONDITIONS

Species	Number of Experiments	Mean Population Doubling Time (+SD)[a] (h)
Rat NTE	2	67 + 1
Rabbit NTE	2	26 + 3
Human NTE	2	61 + 5

[a] Population doubling times were determined from the slope of the exponential portion of the growth curve.



thelium as shown in Fig. 6 *A*. The cells bound antikeratin antibody as evidenced by a positive staining pattern in an immunoperoxidase procedure. Rabbit NTE cells were typically smaller than rat NTE cells but retained an epithelial morphology (Fig. 6 *B*). Human NTE cells were similar in size and morphology to rat NTE cells (Fig. 6 *C*). Cultures of human NTE cells also had areas of multilayering and keratinizing squamous epithelium similar to that seen in rat NTE cell cultures. Rabbit and human NTE cell cultures also stained positively for keratin protein.

Discussion

To study the acute and chronic effects of airborne toxicants on the cells lining the upper respiratory airway, isolation procedures and cell culture methodologies for nasal epithelium were developed. The animals, tissues, and methodology reported herein form the basis of a larger study to extrapolate quantitatively animal inhalation toxicity data to humans. In this report it is demonstrated that various methodologies can be used to (a) isolate NTE cells from a variety of species and (b) grow these cells in long-term culture. The latter finding makes possible future chronic toxicity and carcinogenesis studies with animal and human nasal tissue under controlled in vitro culture conditions.

Three cell isolation procedures were developed: a surgical removal technique followed by enzyme dissociation, an in situ enzyme incubation, and an explant-outgrowth procedure. The first procedure is beneficial for studies involving differential metabolism and cytotoxicity between different areas of the nasal cavity or between species because the cells can be isolated from distinct areas of the nose. This method is operational with rat, rabbit, and human nasal tissue. The in situ incubation method is used for isolating large numbers of nasal epithelial cells from all areas of the rat and rabbit nose for biochemical studies. An advantage of this second method is the extremely low incidence of fibroblast contamination. The explant procedure can be used with all species but has been mainly used with the human tissue to allow continuous isolation of large numbers of turbinate epithelial cells. Although the cells isolated from the explant outgrowths are in culture slightly longer than cells isolated directly from the tissue, the responses to toxicants seem similar between both procedures (unpublished observations).

The hormonal additives used in this study were originally formulated by Dr. Wu et al. (16). Attachment medium was developed only to increase the percentage of cells that would attach to the dish. Although cells from all three species would attach to glass and tissue culture dishes, cell attachment was greatly enhanced in all three species by coating the dishes with a collagen film. A

FIG. 6. Typical morphology of rat (*A*), rabbit (*B*), and human (*C*) nasal turbinate epithelial cells after approximately 10 d in culture. Phase contrast ×438.

number of possible mechanisms could explain this finding: the collagen film may (a) provide for more sites for cell attachment, (b) influence the response to growth factors, (c) enhance growth by modifying cell shape, or (d) provide a diffusible layer that allows nutrients and growth factors to reach the underside of the cell surface. In seeding density experiments the overall efficiency increased as the number of cells plated decreased, suggesting an inhibition of CFE at high cell densities. The rat nasal cells were the most efficient colony formers compared to the rabbit or human cells. However, once cell attachment was completed the rabbit cells had the shortest mean population doubling time compared to either rat or human. Recent experiments in which NTE cells were plated with 3T3 feeder cells indicate that 3T3 cells release media-conditioning factors that can increase the growth rate of rat or human cells to the level of the rabbit cells.

The data indicate that the cells of rats and humans respond differently to FBS, BSA, and calcium levels. The growth of rat NTE cells is stimulated by the addition of either FBS or BSA. However the growth of the human NTE cells is severely inhibited by FBS or moderately inhibited by BSA.

Both rat and rabbit cells seemed to have negligible growth in low calcium or calcium-free media. Rabbit NTE cells grew optimally at higher calcium concentrations than did rat NTE cells. In contrast, human NTE cells grew at a significantly higher rate in low calcium or calcium-free media than did rabbit or rat NTE cells. In some cases, human NTE cells grew optimally at tenfold lower calcium concentrations than did rabbit NTE cells and threefold lower than rat cells, although different specimens of human tissue gave slightly different responses. This lower requirement for calcium in the medium may reflect increased efficiency of uptake or more efficient intracellular utilization by the human cells. High calcium concentrations were severely toxic to rat and rabbit NTE cells. In contrast the same concentrations decreased but did not prevent the growth of human cells.

The data presented here demonstrate that potentially important differences exist in the growth response of NTE cells from various species isolated and grown under the same conditions. Thus it provides additional evidence of the speculative nature of current animal to human extrapolations. These studies make possible future work to predict accurately human response to environmental toxicants from data generated with controlled animal studies.

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